THE L-AMINO ACID OXIDASE OF NEUROSPORA*

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In 1944 one of us described a D-amino acid oxidase in extracts of *Neurospora crassa* (1). Except for slight activity against L-glutamate, no oxidation of L-amino acids was observed. Recently a means for inducing the formation of a soluble L-amino acid oxidase by the mold was reported by Bender, Krebs, and Horowitz (2). This is accomplished by reducing the biotin content of the basal medium (3) from the 5 γ per liter usually employed to 0.25 γ per liter. When cultured in such a medium, *Neurospora* produces an active L-amino acid oxidase which can be demonstrated both in extracts and in the medium. Reduction of the biotin level produces no effect on the D-amino acid oxidase, which is still found in extracts but not in the culture medium. The activity of the L-oxidase toward thirty-eight amino acids has been investigated by Bender and Krebs (4).

The initial purpose of the present investigation was to explore certain possibilities for a genetic study of the enzyme. Of special interest was the fact, cited by Bender *et al.* (2), that the oxidase could not be detected in all of the wild type strains tested. The question arose as to whether this is due to the genetic inability of certain strains to form the enzyme. In connection with the investigation of this problem a general survey of the properties of the enzyme was carried out, together with a preliminary study of the mechanism of the biotin effect and of the adaptive formation of the enzyme. The results of these studies are reported below.

Simultaneously with our investigation and independently of it, Dr. K. Burton (5), working in Professor Krebs' laboratory, has carried out a study of the *Neurospora* L-amino acid oxidase. Where our respective studies overlap mutual confirmation was obtained in most essential points. We wish to thank Dr. Burton for permission to read his manuscript before publication.

Materials and Methods

Cultures—The strains of *N. crassa* used in these experiments include the following wild types: 4A, 1A, 25a, 8a, 12a, Em-5256A, Em-5297a, Lin-

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degren-a, and Abbot-a. They were maintained on slants of the complete medium described by Horowitz (3). Cultures to be used for the preparation of extracts were grown for 5 to 12 days in 500 ml. of minimal medium (3) in Fernbach flasks, or in 200 ml. in 750 ml. Erlenmeyer flasks. To obtain the enzyme from the medium, the mold was grown in smaller volumes (200 ml. per Fernbach flask) for at least 21 days. In either case the biotin content of the medium was limited as noted above. Growth experiments were carried out in 125 ml. Erlenmeyer flasks containing 20 ml. of medium. All cultures were grown at 25°.

Preparation of Enzyme—L-Amino acid oxidase from the culture medium was prepared by filtering the medium from 3 to 4 week-old cultures through Whatman No. 1 paper, chilling the clear filtrate in an ice bath, and adding solid ammonium sulfate to 0.8 saturation. The precipitate, containing the enzyme, was centrifuged and resuspended in 5 ml. of water or buffer for each 300 ml. of the original filtrate. Such preparations were used with or without previous dialysis against cold M/60 phosphate at pH 6. Dialysis slightly increases the activity. The concentrated preparations were stored frozen at −20°. Undialyzed preparations retain their activity for several months, whereas after dialysis a noticeable decrease in activity occurs within a few weeks.

To obtain active extracts, mycelial pads were washed with water on a Büchner funnel and ground with sand and 2 ml. of cold M/60 buffer per gm. of fresh weight of mycelium. After centrifugation, 0.25 volume of 0.2 M buffer was added to the supernatant, and this was used directly. In some experiments the supernatant was first treated with ammonium sulfate at 0.8 saturation and the precipitated enzyme resuspended in M/15 buffer before use.

Unless otherwise noted, enzyme precipitated from the medium was used in the experiments. This preparation is considerably purer than that obtained by extraction of the cells and shows neither the background oxygen uptake nor the initial lag in L-amino acid oxidase activity which is observed in extracts.

EXPERIMENTAL

Determinations of enzyme activity were made in the Warburg apparatus at 30°. 0.25 ml. of enzyme concentrated from the medium plus 1.75 ml. of M/15 buffer (pH 6 unless otherwise noted) was used per vessel. When extracts were tested, 2 ml. of the crude or purified extract were used in each vessel. The side arm contained 0.25 ml. of substrate M/30 with respect to the L isomer. Alkali was used in the center well, and the atmosphere was oxygen.

When activity is expressed as QO_2, this represents microliters of O_2 per hour per mg. of protein. Protein was determined gravimetrically after

immersion of the solution in boiling water for 3 minutes, followed by chilling, centrifugation, and washing. The washed precipitates were dried overnight at 90° in tared beakers.

Properties of Enzyme

Stoichiometry—With L-alanine as substrate, simultaneous determinations of oxygen consumption and of ammonia and pyruvate production were made. Ammonia was determined by nesslerization after distillation by the Conway-Byrne technique. Pyruvate was measured by the "direct method" of Friedemann and Haugen (6). The results are shown in Table I. It is seen that in the over-all reaction 1 atom of oxygen is consumed and 1 molecule each of ammonia and pyruvate is produced per molecule of alanine oxidized. As noted previously (2), the preparations contain cata-

TABLE I

Oxidation of L-Alanine; Stoichiometric Relations

Each vessel contained 0.25 ml. of M/30 L-alanine, 0.5 ml. of dialyzed enzyme, and 1.5 ml. of M/15 phosphate buffer, pH 6.

Vessel No.	O_2 Consumed		Pyruvate produced			Ammonia produced		
	$\mu l.$	μM	γ	μM	μM *per* μM O_2	γ N	μM	μM *per* μM O_2
1	83	3.7	680	7.7	2.08	122	8.7	2.35
2	92	4.1	750	8.5	2.07	118	8.4	2.04
3	91	4.1	705	8.0	1.95	114	8.1	1.97
Mean....					2.03			2.12

lase; thus hydrogen peroxide does not accumulate. That peroxide is produced, however, is indicated by the fact that the addition of ethanol to the reaction mixture causes an increase in the oxygen uptake (7). In two such experiments, with L-leucine as substrate, the oxygen uptake was, respectively, 1.33 and 1.64 atoms per molecule oxidized. No oxidation of ethanol occurred in the absence of an amino acid substrate.

pH Optima—The effect of pH on the activity of the oxidase was determined in the range pH 5.6 to 7.6 with phosphate buffers. Data obtained with five different substrates are presented in Fig. 1. In the case of ornithine, the oxidation rate is only slightly affected by pH changes in the range studied, whereas with glutamate an optimum occurs at pH 6.8. Oxidation of monoaminomonocarboxylic acids is also strongly pH-dependent, with optima at pH 7.2 or higher. These results indicate that the state of ionization of the substrate is important for activity, a supposition which is strengthened by the observation that esterification of the carboxyl group of histidine abolishes its reactivity (Table III).

Except when stated otherwise, our experiments were carried out at

pH 6.0. This pH approximates that of freshly prepared *Neurospora* extracts.

Enzyme and Substrate Concentration Effects—The initial rate of oxidation is linear with respect to enzyme concentration (Fig. 2).

When the rate of oxidation is plotted against substrate concentration, an optimal concentration is observed (Fig. 2). A similar phenomenon has been reported for the L-amino acid oxidase of snake venoms (8, 9). The

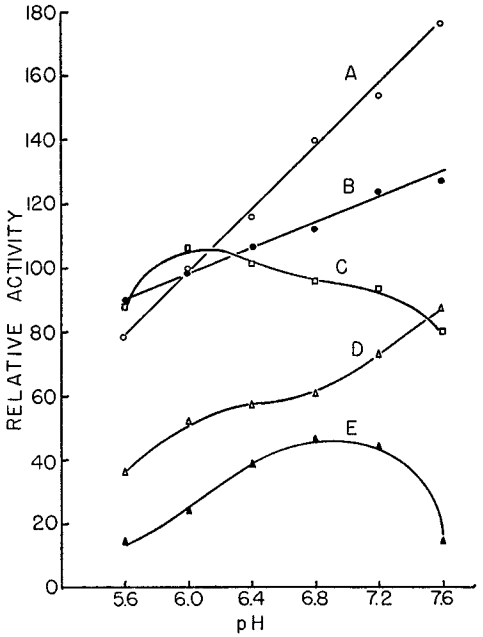

FIG. 1. Effect of pH on activity of *Neurospora* L-oxidase. The ordinates are relative rates based on leucine at pH 6 as 100. Curve A, L-leucine; Curve B, L-phenylalanine; Curve C, DL-ornithine; Curve D, L-methionine; and Curve E, L-glutamate.

Neurospora system further resembles that of snake venom (8) in showing interference between two substrates added to the enzyme simultaneously. In Table II are presented initial oxidation rates of a number of amino acids tested singly and in pairs. It is seen that the less rapidly oxidized amino acids inhibit the oxidation of the more rapidly oxidized ones. This result is most simply interpreted on the basis of competition between the substrates for the enzyme surface. It was previously demonstrated that isovaline (α-methyl-α-amino-n-butyric acid) competitively inhibits the oxidation of methionine by the D-amino acid oxidase of *Neurospora* (1).

Oxygen Effect—An increase in enzymatic activity of 3- to 6-fold (average

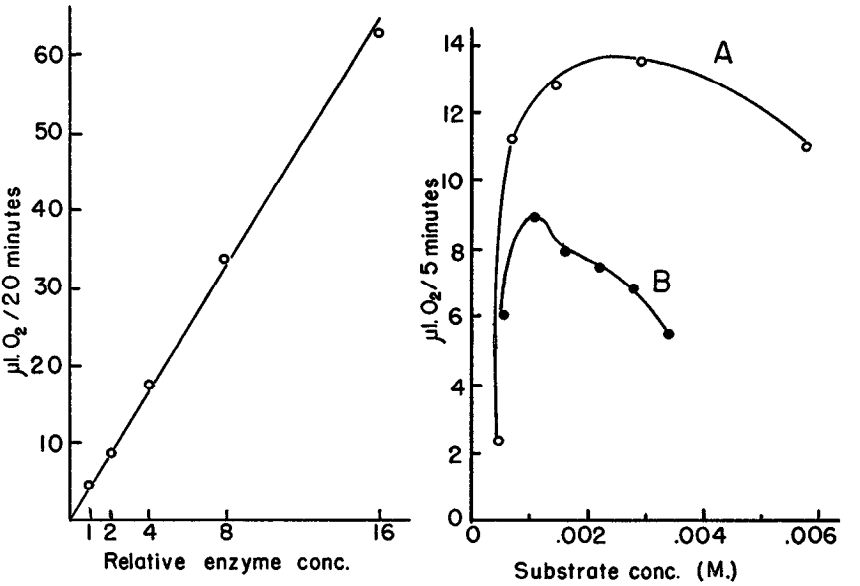

FIG. 2. Enzyme and substrate concentration curves. Left, serial dilutions of enzyme in 0.044 M phosphate, pH 6.0; substrate, L-canavanine. Right, substrate dilutions. Curve A, L-leucine in 0.053 M pyrophosphate, pH 8.5; Curve B, L-canavanine in 0.052 M phosphate, pH 6.

TABLE II

Competition between Mixed Substrates

Experiment No.	pH	Substrate	O_2 consumed in 1st 10 min.
			µl.
1	7.6	M/300 DL-isovaline	0
		M/600 L-phenylalanine	42
		M/600 " + M/24 DL-isovaline	30
2	5.6	M/600 L-lysine	42
		M/600 " + M/24 DL-isovaline	19
3	6.0	M/540 L-arginine	31*
		M/270 "	20*
		M/540 L-lysine	30*
		M/270 "	15*
		M/540 " + M/540 L-arginine	17*
4	6.0	M/665 L-canavanine	27
		M/665 " + M/300 L-glutamate	21
		M/665 " + M/300 L-aspartate	20
		M/665 " + M/300 L-methionine	10
		M/665 " + M/300 L-lysine	5

* O_2 consumed in 20 minutes.

5-fold) is observed when the tests are carried out in oxygen as compared to air. A similar effect has been reported for the L-amino acid oxidase of moccasin venom in a recent paper by Singer and Kearney (9).

Specificity—As noted previously (4), the enzyme attacks only L-amino acids. In Table III are shown the relative rates of oxidation of thirty-three amino acids, based on L-leucine as 100. The QO_2 of leucine varied in different experiments from 245 to 375, with a mean of 320. Correction for the variation in absolute activity among different enzyme preparations was made by standardizing each preparation on L-leucine.

The relative reactivities of the amino acids shown in Table III differ somewhat from those reported by Bender and Krebs (4), particularly in the cases of histidine, lysine, and ornithine. These and other differences are undoubtedly due, for the most part, to the fact that we have worked at pH 6, whereas the Sheffield workers made their measurements at pH 8.4. The basic amino acids are nearer their optima at the lower pH, while the reverse is true for monoaminomonocarboxylic acids.

As observed above, the reaction consumes 1 atom of oxygen per mole of substrate. The following exceptions to this rule were noted: tyrosine, glutamine, cystathionine, ornithine, and canavanine. (Because of their slow rates of reaction, the oxidations of glutamate, alanine, isoleucine, valine, and diaminobutyric acid were not run to completion.) Tyrosine consumes in excess of the theoretical value (>1.8 atoms when the reaction was stopped), a fact also noted by Bender and Krebs (4). The solution turns red and then black in the course of the reaction, indicating the presence of tyrosinase in the enzyme preparation. Glutamine consumed only 0.83 atom of oxygen, suggesting that the amide may have been partially hydrolyzed to the slow reacting acid. L-Cystathionine, containing two reactive amino groups, took up 1.3 atoms instead of the expected 2 atoms, while canavanine and ornithine consumed, in numerous trials, 0.8 to 0.9 and 0.75 to 0.85 atom, respectively. Partial oxidation (0.7 atom) of ornithine was also obtained by Bender and Krebs. The following compounds were not attacked at a measurable rate: glycine, DL-serine, DL-threonine, L-proline, L-aspartate, DL-isovaline (α-amino-α-methyl-n-butyric acid), DL-lactate, and L-histidine methyl ester.

Production of Enzyme

Strain Differences—The results of Bender *et al.* (2) had indicated that under identical conditions some wild type strains of *Neurospora* produce only a D-amino acid oxidase, some only an L-amino acid oxidase, while others form both enzymes. Further investigation, described below, has shown that, while quantitative differences probably exist among the strains with respect to production of the enzymes, there is no absolute difference

among them, and both oxidases can be demonstrated in every strain which we have tested.

Crude extracts of *Neurospora* cultured in a low biotin medium show a high background rate of oxidation as compared to extracts prepared from cultures grown in the standard medium. This oxidation may remain constant in rate for periods of time varying from a few minutes to several

TABLE III

Rates of Oxidation of L-*Amino Acids by Neurospora* L-*Oxidase*

All rates are expressed relative to L-leucine as 100. L isomer concentration M/270 in all cases except L-cystine (M/540). Oxygen uptake measured in first 10 minutes; pH 6.0.

Substrate	Relative activity	Substrate	Relative activity
L-Histidine	140	L-Methionine	53
DL-α-Amino-*n*-butyric acid	134	L-Tryptophan	43
L-Canavanine	125	L-Lysine	40
L-Tyrosine	122*	DL-α-Amino-ε-hydroxy-*n*-caproic acid†	38
DL-Ornithine	106	L-α,γ-Diaminobutyric acid	35
DL-Phenylalanine	106	L-Alanine	32
L-Leucine	100	L-Glutamic acid	24
L-Phenylalanine	99	L-Valine	19
DL-α-Aminoadipic acid†	96	DL-Isoleucine	18
L-Cysteine	85	Glycine	0
L-Cystine	81	DL-Serine	0
DL-α-Amino-*n*-valeric acid	80	DL-Threonine	0
DL-Citrulline	79	L-Proline	0
L-Glutamine	64	DL-Aspartic acid	0
DL-Norleucine	61	DL-Isovaline	0
L-Cystathionine	58	L-Histidine methyl ester	0
L-Arginine	56	DL-Lactic acid	0

* Includes tyrosinase activity (see the text).

† Compound furnished through the courtesy of Mr. Norman Good.

hours before it diminishes. If an L-amino acid is added during the constant period, no increase in the rate of oxidation is observed, but the decline in rate is postponed. If, however, the background rate is allowed to fall off somewhat before the amino acid is added, then an immediate increase in oxygen uptake is obtained (Fig. 3). In either case, an extra quantity of oxygen is consumed equivalent to the substrate added. These results suggest that the background oxygen consumption of the extracts is due chiefly to the oxidation of endogenous L-amino acids which, at the outset, saturate the system so that further addition of L-amino acids is

without effect on the oxidation rate. This conclusion is supported by the following evidence: (1) *Neurospora* extracts contain 8 to 35 μM of free amino

TABLE IV

Effect of Various Cultural Conditions on Activity of L-*Oxidase*

All experiments were performed with 2 ml. of enzyme solution and 0.25 ml. of M/30 L-leucine as substrate. The enzyme was prepared by precipitation from extracts. Oxygen consumption was measured in the first 10 minute period after mixing.

Experiment No.	Culture medium	Age	pH of reaction mixture	Activity	
				Per gm. fresh mycelium	Per mg. protein
		days		*μl. per hr.*	*μl. per hr.*
1	Minimal, high biotin	7	7	35	1.2
	" low "			128	5.6
2	N-free minimal, high biotin + 0.525% $(NH_4)_2SO_4$	7	6	11	0.4
	N-free minimal, high biotin + 0.25% $(NH_4)_2SO_4$			50	2.9
3	Minimal, low biotin	7	6	48	1.2
	" " " + 0.1% casein hydrolysate*			373	8.8
	Minimal, low biotin + 0.25% casein hydrolysate			372	10.7
	Minimal, low biotin + 0.5% casein hydrolysate			614	12.1
4	Minimal, low biotin	9	6	43	
	" " " + 0.1% casein hydrolysate			80	
	Minimal, low biotin + 0.15% L-glutamate			40	
	" " " + 0.01% L-leucine			32	
	" " " + 0.15% "			138	
5	" " "	5	6	34	2.2
	" " " + 0.15% L-leucine			188	7.3
	" " " + 0.195% L-lysine HCl			330	16.8
6	N-free minimal, low biotin + 0.42% NH_4Cl			45	3.7
	" " " " + 0.28% " + 0.24% casein hydrolysate	5	8	333	32.8
	N-free minimal, low biotin + 0.14% NH_4Cl + 0.48% casein hydrolysate			420	37.6
	N-free minimal, low biotin + 0.72% casein hydrolysate			635	47.2

* "Vitamin-free" casein hydrolysate, Nutritional Biochemicals Corporation.

acids per gm. of fresh mycelium, as determined by the colorimetric ninhydrin method (10). The presence of alanine, methionine (or valine), the leucines (or phenylalanine), and others has been demonstrated by paper

chromatography. (See also Fling and Horowitz (11).) (2) Extracts which have been purified by ammonium sulfate precipitation or dialysis show immediate oxidation of added L-amino acids with little or no background oxygen consumption. (3) If, to a boiled low biotin extract, some highly active L-oxidase precipitated from the culture medium is added, a quantity of oxygen is consumed which approximates that consumed by the crude unboiled extract. (4) The addition of a D-amino acid to crude extracts

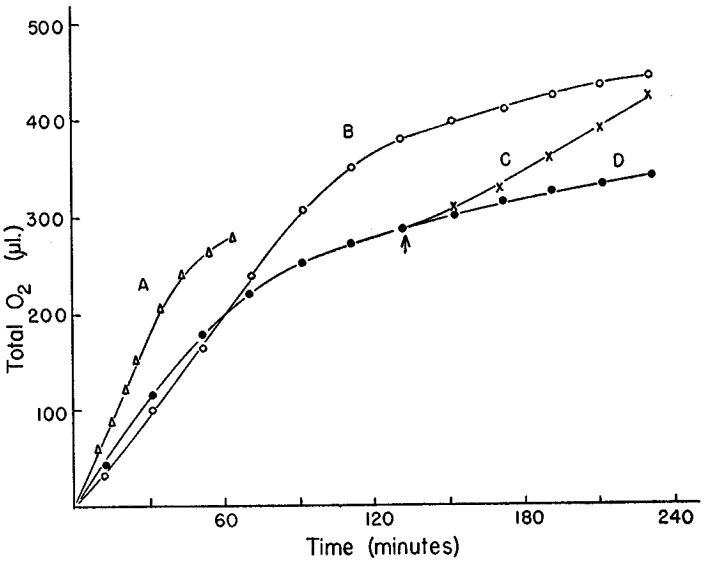

FIG. 3. Effect of immediate and delayed addition of amino acids on O_2 uptake of a crude extract. Curve A, D-methionine added at time zero; Curve B, L-phenylalanine added at time zero; Curve C, L-phenylalanine added at 132 minutes (arrow); Curve D, endogenous control, no substrate added. pH 8.0.

during the constant phase of the endogenous respiration results in an immediate increase in the oxidation rate (Fig. 3).

With the above facts in mind, a reinvestigation was made of strains which, on the basis of the earlier tests, had appeared to be devoid of the L-oxidase. In every case it was found that the enzyme is easily demonstrated after the background oxidation enters the diminishing phase. At the same time, it was found that the enzyme could be detected in the medium (low biotin) of all tested strains if the enzyme is concentrated by precipitation and resuspension in a smaller volume as described above.

The question of the non-occurrence of the D-amino acid oxidase in certain strains has also been reinvestigated by us and by Dr. K. Burton in Professor H. A. Krebs' laboratory. Burton has found that the D-oxidase is

demonstrable in all the strains examined if the tests are carried out on young cultures, but that it disappears from old cultures ((5) and personal communication from H. A. Krebs). Our results are in agreement with this finding and show that the enzyme is regularly present in 7 to 10 day-old cultures of the various strains used. It is thus very likely that the previously reported failure to find the D-oxidase in certain strains was due to the ages of the cultures used.

Biotin Effect—Preliminary experiments have been carried out to elucidate the mechanism of the biotin effect on L-oxidase production. It was first of interest to determine whether growth of the mold is actually limited by biotin in the low biotin medium. Growth of wild type strain 8a was therefore determined as a function of the biotin concentration, growth being measured after 72 hours at 25°. It was found that the growth curve rises steeply as the biotin concentration increases, reaching a plateau in the neighborhood of 0.5 γ of biotin per liter. Beyond this point, further addition of biotin up to at least 2 γ per liter had no effect on growth. At a concentration of 0.25 γ per liter (the concentration of biotin in the "low biotin" medium) growth was 85 per cent of maximal. In experiments with other strains in which growth at a biotin concentration of 0.25 γ per liter was compared with that at 5 γ per liter, it was found that growth is frequently maximal at the lower concentration, even for growth periods as long as 2 weeks. It is concluded that 0.25 γ of biotin per liter is neither much in excess of the requirement for normal growth nor is it seriously limiting. It can be described as marginal. This is in essential agreement with previous work on the biotin requirement of *Neurospora* by Ryan, Beadle, and Tatum (12) and by Hodson (13).

It was desirable to learn whether cultures grown in the standard high biotin medium are actually devoid of measurable L-oxidase activity, or whether the failure to demonstrate it in extracts of such cultures is the result of dilution, fractionation, or the masking effect of endogenous amino acids described in the previous section. Extracts of high biotin cultures were therefore purified by precipitation in 0.8 saturated ammonium sulfate and by dialysis. Such preparations showed a low and variable, but significant, L-oxidase activity (Table IV). At the same time, the washed, insoluble residue resulting from the extraction was tested and was also found to exhibit a small activity. It is not known whether this activity is due to particle-bound enzyme or to enzyme contained in intact cells in the residue. It is evident that even at high biotin levels *Neurospora* is not completely devoid of L-amino acid oxidase.

On the assumption that the enzyme is a flavoprotein (see Burton (5)), we have investigated the possibility that biotin controls production of the enzyme by participating in a system which destroys riboflavin. If this

were the case, it might be expected that addition of riboflavin to the medium would have an effect on L-oxidase formation similar to that obtained by reduction of the biotin content. No effect on L-oxidase production was found, however, in experiments in which the mold was grown on both standard and biotin-deficient media supplemented with 0.5 to 10 mg. of riboflavin per liter. The addition of a boiled L-oxidase preparation to high biotin extracts did not increase their activity toward L-amino acids.

Another series of experiments was designed to ascertain whether limiting growth by reducing the concentration of other constituents of the medium influences L-oxidase formation. Tests were carried out with sulfur- and nitrogen-deficient media. No effect was found in sulfur-deficient media, but an effect comparable to that obtained by limiting biotin was obtained by growing the organism in a nitrogen-deficient medium. In the sulfur experiment a mutant, No. 85518, which is unable to reduce sulfate, but which grows readily when supplied thiosulfate, was employed. No increase in L-oxidase activity was detected in extracts of this strain grown on a limiting concentration of thiosulfate (0.75×10^{-4} M). In the nitrogen experiment a wild type was used. It was grown for 6 days in high biotin media containing 0.53 and 1.11 gm. of ammonium nitrogen per liter. Growth is limited only slightly at the former concentration, while the latter supplies an amount of nitrogen equal to that in the standard minimal medium. Determinations of L-oxidase activity in extracts showed 4 to 7 times more activity in the low N culture than in the control (Table IV). The nitrogen effect has not been obtained consistently and is being investigated further.

Adaptive Formation of L-*Oxidase*—Even higher activities were obtained when casein hydrolysate was added to low biotin medium containing the usual amounts of ammonia and nitrate, or when casein hydrolysate was substituted for part or all of the inorganic nitrogen (Table IV).

To determine whether this adaptive effect is due to any particular amino acid, leucine, lysine, and glutamic acid were added singly to low biotin minimal medium which was then used for culture of the organism. The results (Table IV) show that the first two, which are good substrates for the enzyme, reproduce the effect of casein hydrolysate, while glutamic acid, the major amino acid of casein but a poor substrate for the enzyme, does not. It is concluded that the adaptive response is probably elicited by any amino acid which is readily attacked by the enzyme. It is of interest to note that the leucine- and lysine-induced enzymes showed no differences in their respective activities toward leucine and lysine.

It is significant that adaptive production of the enzyme does not occur in a high biotin medium, even when casein hydrolysate is the sole source of nitrogen.

DISCUSSION

The data presented above show that wild type *N. crassa* produces an L-amino acid oxidase, part of which is soluble and part of which may be bound to the insoluble constituents of the cell. The amount of enzyme in the cells depends on cultural conditions and probably also on the inherent properties of the strain, but no strain we have tested is devoid of the enzyme.

Quantitative estimation of the oxidase in extracts requires that the latter first be freed of amino acids which are present in the extracts of both high and low biotin cultures and which partially or completely saturate the enzyme. In extracts with a relatively high L-oxidase content the enzymatic activity itself soon lowers the endogenous amino acid concentration to a point at which the addition of an exogenous amino acid results in an immediate increase in oxidation rate. In extracts of low activity, this may require several hours or may never be realized in practice. In the latter case it is necessary to purify the extract by precipitation or by dialysis, in order to demonstrate the presence of the enzyme. The masking effect of endogenous amino acids, together with the interference effect described above, accounts for the previous failure to demonstrate the enzyme in crude extracts of certain strains and is responsible for the lag in L-oxidase activity which is observed in such preparations.

L-Oxidase activity is increased 4- to 10-fold in extracts of cultures which have been grown on a marginal biotin supply and is further increased by the addition of amino acids to marginal biotin medium. The interrelationship of the various factors controlling L-oxidase production is not yet understood, but a connection between biotin and nitrogen metabolism and between biotin and adaptive enzyme formation may be inferred. It was observed a number of years ago by Winzler, Burk, and du Vigneaud (14) that biotin is necessary for the rapid absorption of ammonia by yeast. There is therefore reason for believing that biotin limitation may to some extent be equivalent in metabolism to nitrogen limitation. In several experiments we have found an apparent increase in L-oxidase activity in cultures grown on nitrogen-deficient media. However, the variable occurrence of this response requires clarification. It is of interest to note the finding by Nason, Kaplan, and Colowick (15, 16) that the concentration of another enzyme, diphosphopyridine nucleotidase, also increases in *Neurospora* under biotin or nitrogen deficiency, and under zinc deficiency as well. Also of interest is the finding by Blanchard *et al.* (17) that biotin is needed for the adaptive formation of the "malic" enzyme of *Lactobacillus arabinosus*.

Our results indicate that L-amino acid oxidase production in *Neurospora* may be an adaptive process similar to adaptive enzyme formation in other

microorganisms, and, further, that the adaptation is prevented when an excess of biotin is present in the medium. On this basis, the increased production of the enzyme under conditions of limited biotin supply can be accounted for by the hypothesis that under these conditions adaptation occurs toward endogenous amino acids.

SUMMARY

Neurospora crassa produces an L-amino acid oxidase which oxidatively deaminates a large number of L-amino acids to the corresponding keto acids and ammonia. Production of the enzyme is greatly increased by culturing the organisms on media containing a marginal supply of biotin. If amino acids are added to a low biotin medium, a further increase in enzyme production is elicited. This response does not occur in a high biotin medium. These results suggest a connection between biotin, nitrogen metabolism, and adaptive enzyme formation.

The enzyme is 3 to 6 times more active in oxygen than in air. The pH optimum varies with the amino acid used as substrate. The enzyme is inhibited at high substrate concentrations, and the simultaneous addition of two amino acids results in an interference effect.

By taking certain precautions the presence of both the L- and the D-amino acid oxidases has been demonstrated in all of the strains of *Neurospora* tested.

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